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BY

GEORGIA WINIFRED BURTON

DISSERTATION

Submitted in Partial Fulfillment of the Requirements
for the Degree of Doctor of Philosophy in
the Faculty of Pure Science of
Columbia University.

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EFFECT OF HYDROGEN ION CONCENTRATION UPON THE DESTRUCTION OF VITAMIN B BY HEAT

Vitamin B is perhaps the most stable of the known vitamins. It has long been thought to be relatively stable to oxidation, as well as to heat, and to be more stable in acid than in alkaline solution.

As early as 1909, Fraser and Stanton[1] began their extensive study of beriberi and its causes. In 1911, they announced that the disease was caused by the consumption of polished rice as a staple diet. These experimenters found that the substance or substances in rice polishings or in unpolished rice which prevented beriberi, was "soluble in alcohol and was decomposed by sodium hydrate." In 1915, they[2] concluded that a 5 per cent. sodium hydroxide solution would destroy the active substance.

Drummond [3] in 1917, announced that he had found "marmite," after being treated with a five per cent. sodium hydroxide solution at room temperature, to show very little depreciation of its growth-promoting factor. If the same substance was treated with hot five per cent. sodium hydroxide, the destruction tended to be complete.

McCollum and Simmonds[4], in the same year, extracted the active factor from ether-extracted wheat embryo by the use of 95 per cent. alcohol for three consecutive six hour periods. After distilling off the alcohol, the extract was taken up in water. The solution was neutralized to phenolphthalein with sodium hydroxide and an excess of sodium hydroxide added to make it alkaline up to 0.28 per cent. NaOH. After heating one hour under a reflux, it was neutralized with hydrochloric acid, and acidified by an addition of 12 per cent. acetic acid. This solution was evaporated, and the dried residue fed to rats. The workers concluded from their results that the water-

soluble vitamin B in the wheat embryo was almost completely destroyed by the heating with the sodium hydroxide.

Osborne and Leavenworth [5] in 1920 treated a yeast preparation with sodium hydroxide for (1) ½ hour at 20° C, (2) 18 hours at 20° C, (3) 90 hours at room temperature, and (4) 18 hours at room temperature followed by one hour at 90° C, re-acidified and made into tablets. These investigators found from their results that "in an attempt to concentrate or isolate the water-soluble vitamin B, dilute alkaline solutions can be used without materially affecting its activity provided a low temperature and a short time of exposure to the alkali are employed."

The early studies in this field, however, have been for the most part qualitative in nature. In 1923, Sherman and Edgeworth [6] made a careful study of the rat-growth method for the determination of the relative amounts of vitamin B. They demonstrated the fact that reliable quantitative results could be obtained by a careful comparison of the weight curves of rats fed upon a purified vitamin B-free diet to which a measured amount of milk powder as a source of vitamin B, had been added. Sherman and Spohn[7], using this method, found vitamin B to be more stable in the dry than in the liquid state. These investigators found no measureable dimunition of vitamin B in milk powder heated dry in air at 100° C for 48 hours, but a destruction of 25 per cent. when the liquid milk was heated six hours at the same temperature. Investigations were made by Sherman and Grose[8] using the rat as the experimental animal in order to determine quantitatively the stability of vitamin B in solution at different temperatures. They found a destruction of 20 per cent. of the vitamin when tomato juice was heated 4 hours at 100° C, 33 per cent. when heated 4 hours at 110° C, 47 per cent. when heated 4 hours at 120° C, and 55 per cent. when heated 4 hours at 130° C.

The present investigation was undertaken to determine quantitatively, if possible, the rate of destruction of vitamin B by heat under several conditions of alkalinity. Upon the suggestion of the work of Sherman and Edgeworth [6], the rat-growth method was used throughout the investigation.

EXPERIMENTAL

General Method. The method used in this investigation is essentially as follows: Young growing albino rats, twenty-eight days old, were given a diet adequate in every factor except the vitamin to be tested. Different doses of tomato juice, which had previously been subjected to heat at different concentrations of hydrogen ion, were added to the basal diet and the increase, or decrease, of body weight determined as an indication of the vitamin present.

Selection and Care of Animals. The animals were normal young albino rats. These animals were placed on weaning, at the age of twenty-eight or twenty-nine days, in individual round cages with false bottoms. They were given the experimental diet *ad libitum*, together with fresh distilled water. The tomato juice was carefully measured by means of a graduated pipette into a small feeding cup and placed in the cage at a nearly uniform hour each day except Sunday. The mothers of the rats used, had been raised upon one of the several adequate stock diets of wheat and milk powder with a possible addition of lemon juice and butter. A positive control, a rat given tomato juice heated at natural acidity, (pH = 4.28), was kept in parallel with each experimental animal receiving tomato juice heated at a lower concentration of hydrogen ion. In this way, it was hoped to eliminate any variations due to previous dietary history of the animals, or to changes in season. Animals whose initial weights were between 38 and 50 grams, were selected whenever possible (in no case was the initial weight less than 33 nor more than 63 grams). Each animal was weighed when put on the experimental diet, and at intervals of seven days thereafter. A record was kept of the food eaten each week. Cages of rats used in comparisons were kept close together in order to insure like conditions of light and heat.

Basal Diet. Diet 107, as described by Sherman and Spohn[7], was used throughout this investigation. This diet was shown by them to furnish practically optimum amounts and proportions of all nutrients required by the normal rat except vita-

min B. All increases in growth of rats given tomato juice could be attributed to vitamin B in the latter. The composition of the diet used was:

Extracted casein - - - - - -	18 per cent.
Salt mixture (Osborne and Mendel[9])	4 per cent.
Butter fat - - - - - - - -	8 per cent.
Cod liver oil - - - - - - -	2 per cent.
Corn starch - - - - - - -	68 per cent.
	100 per cent.

The casein was freed from its vitamin B by careful cold extraction, twice with 60 per cent. (by weight) alcohol, and once with 90 per cent. alcohol, according to the method described by Sherman and Spohn[7]. The butter fat used was first freed from curd, water, and salt, by melting below 50° C, solidifying and separating carefully. This process was repeated, and the fat was finally filtered through a qualitative paper within a hot-water funnel. Destruction of vitamin A was avoided by keeping the temperature of the fat at all times below 50° C. The cod liver oil used was a Squibb's product. The salt mixture was prepared according to the direction of Osborne and Mendel[9]. The corn starch was a commercial product shown to be free from vitamin B by Osborne, Wakeman and Ferry[10]. All ingredients of the basal diet were very thoroughly mixed by hand.

Preparation of Vitamin B Material. The canned tomatoes were bought at intervals, but they were all of the same brand and canned the same season. The contents of the can were thrown on cheese cloth, and allowed to filter at 10° C. The juice was then passed through a qualitative filter and finally through Schleicher and Schull quantitative filter paper No. 590. In this way, a clear yellow liquid was obtained.

The heating at 100° C was carried out according to the method used by Sherman and Grose[8]. Erlenmeyer flasks containing the tomato juice were immersed to the necks in a bath of boiling water heated by steam. Loosely fitting glass stoppers with flanges that rested upon the tops of the flasks were

used to render the evaporation negligible. Upon the completion of the accurately timed heating, the flasks were plunged under a tap of cold water. The time required for cooling was practically equivalent to that required for the tomato juice to reach the temperature 0.3° or 0.4° C., below that of boiling water, and therefore, any error introduced was negligible.

In order to test the effect of the hydroxyl ions upon the stability of vitamin B, samples of tomato juice at different degrees of hydrogen ion activity (pH) were prepared. Tomato juice was found to have a natural hydrogen ion activity of from pH = 4.12 to pH = 4.34. This is in close agreement with the results given by LaMer, Campbell and Sherman[11], and by Clark and Lubs[12]. After the juice had been heated for one hour the activity of the hydrogen ion was practically unchanged, (pH = 4.14 to pH = 4.36). In those samples of juice to which alkali had been added, the pH decreased during the heating. The yellow color of the tomato juice was turned to a reddish brown during the heating (of the alkalinized samples), and this dark color was probably due to humin formation or the caramelization of the sugars. Electrometric determinations of pH in the various preparations before and after heating gave the following results: Preparation I, 5.20, 5.02; II, 7.90, 6.96; III, 9.18, 8.01; IV, 10.9, 8.5; V, 5.21, 4.98; VI, 7.90, 6.24.

In all cases, practically 0.4 normal sodium hydroxide was used to lower the concentration of the hydrogen ion. The volume of the mixture, tomato juice, alkali and acid, equivalent to one cubic centimeter of natural tomato juice was calculated for each preparation. As the tomato juice was to be fed as sole addition to an otherwise constant basal diet, the introduction of salts to "buffer" the solutions was precluded by the nature of the investigation.

The alkali was added dropwise, and care was taken to keep both liquids below 10° C. during the additions in order to minimize any changes which might tend to result from local excess. From 20 to 25 cubic centimeters of the mixture was brought to room temperature for the hydrogen ion determination. After the heating period, the solution was once

more cooled very quickly to room temperature, and its hydrogen ion concentration determined in a 20 cubic centimeter portion. Hydrochloric acid equivalent to the sodium hydroxide, was added to prevent the effect of the hydroxyl ions continuing after the sample was placed in the ice chest.

The hydrogen ion concentrations were determined electrometrically using hydrogen ion electrode vessels similar to those described by Wilson and Kern[13] and a Leeds and Northrup potentiometer. The solution whose hydrogen ion concentration was to be determined, was poured into a glass container with a small diameter and a capacity of about 30 cubic centimeters. The determinations were carried out at room temperature, 20° C.—23° C., and corrections were made for all changes in temperature by means of the Nernst formula. Barometric pressure corrections were not made since they would necessarily be less than the error of experimentation. A saturated calomel electrode was employed because it had been shown by Fales and Mudge[14] to give a very constant reading over a range of temperatures. Carefully platinized and tested electrodes were used since they have a lower sensitiveness to oxygen and hence a longer life than the bright electrodes[15]. Each electrode was used for two or three readings provided it did not show deterioration when checked against a standardized acid.

In order to determine whether oxidation was a factor in the destruction of vitamin B here studied, additional comparisons were made. Part of the tomato juice brought to a hydrogen ion concentration of pH = 9.18 was heated in loosely stoppered bottles, as described above, while a second part was heated in an atmosphere of nitrogen, according to the method used by Quinn[16].* The first portion was kept at

* Note: In order to insure the highest possible efficiency in freeing the tomato juice from oxygen, the apparatus was tested from time to time. The heating flask was filled with distilled water instead of tomato juice. The apparatus was sealed carefully with shellac and sealing wax as when used for the tomato juice, and nitrogen was passed through it for one hour. The water was then tested for the presence of oxygen by the introduction of indigo white through a side tube. A negative test showed that the dissolved oxygen had been expelled from the water.

room temperature for two hours before heating. In this way, both portions were exposed to the action of the alkali for two hours at room temperature, and one hour at 100° C. The latter portion was cooled, its hydrogen ion concentration determined, and re-acidified as before, but it was kept in the ice chest under an atmosphere of nitrogen. Oxygen was not allowed to come in contact with this latter portion from the time of filtering until it was pipetted into the cups for feeding. Control animals were at the same time fed on tomato juice of natural acidity heated in air or in an atmosphere of nitrogen.

In all of the above work, the rats drank the tomato juice as soon as it was placed within the cage, and hence the loss in feeding was very small. It was necessary for the first two or three days to force those rats fed on tomato juice heated at a very low concentration of hydrogen ion, to drink their tomato juice by leaving them for several hours each day without water. In the case of those rats dying from lack of vitamin B, the dose was occasionally refused a day or even two days before death. In order to avoid possible bacterial decomposition, fresh samples of tomato juice were prepared at short intervals. Each rat, then, received its doses from a large number of samples, and in this way, any possible effects of accidental errors were lessened.

RESULTS

Many animals were used to determine the dosage of tomato juice necessary for maintenance. Growth around the maintenance zone, was found to be proportional to the size of the dose. The rats, as shown by the growth curves, were quite sensitive to slight changes in doses of tomato juice, and since it was not practical to feed large enough daily doses for a definite steady gain in weight during the eight weeks period, comparisons were all made around maintenance, or below.

The comparisons were made between the growth curves of rats fed upon a basal diet together with a measured portion of tomato juice heated at natural acidity, and the growth

curves of rats fed upon the basal diet together with tomato juice heated at a lower concentration of hydrogen ions. It was necessary, then, to establish the destruction of the vitamin B by heat in order to calculate the total destruction at any given hydrogen ion concentration. From the work of Sherman and Grose[8] in this laboratory, approximately 20 per cent. of the vitamin B was shown to be destroyed when tomato juice was heated in a steam bath as previously described for four hours. The limited number of rats used to relate this work and that of the earlier investigation, agree quite closely with the results of Sherman and Grose[8].

In cases of low concentration of hydrogen ion, the loss of the growth promoting factor was so great that it was found necessary to make some comparisons as a measure of destruction due to heat at 100° C. in one hour rather than in four hours. Table I shows the comparison of the gains in weight of rats fed on unheated tomato juice, and on tomato juice heated one hour at 100° C. under conditions of natural acidity (pH = 4.28).

Since the vitamin B requirement may be in direct relationship with the active body weight according to the work of Osborne and Mendel [17], or it may be proportional to the ⅔ power of the weight, the quantity of energy handled as indicated by the total calories utilized from the food, and the body weight[18], an effort was made to keep the average initial weight readily comparable. Any variation caused by rats of different initial weight fed on an equal dosage of tomato juice, is overcome by the use of a positive control of approximately equal initial weight from the same litter.

Table I shows the rat number, the dose of tomato juice, the initial weight, the food intake, and the gain in weight over a period of eight weeks, for a group of rats with their exactly parallel positive controls. From the curves in Figure I it may be seen that 5.5 cubic centimeters of tomato juice heated for one hour at natural acidity (pH = 4.28) is approximately equivalent to 5 cubic centimeters of unheated tomato juice.

TABLE I.

RATS FED ON UNHEATED TOMATO JUICE AND ON TOMATO JUICE HEATED ONE HOUR AT 100° C. BOTH AT NATURAL ACIDITY (pH = 4.28).

Rat No.	Initial Weight gms.	Dose cc.	Food Intake gms.	Gain gms.	Rat No.	Initial Weight gms.	Dose cc.	Food Intake gms.	Gain gms.
16118	48	5	237	6	16119	46	5.5	241	1
16122	36	5	237	5	16120	43	5.5	194	–2
16124	49	5	224	5	16121	37	5.5	220	3
16125	47	5	212	3	16123	52	5.5	226	4
16127	45	5	278	10	16126	33	5.5	221	12
16129	37	5	236	6	16128	43	5.5	257	3
16130	61	5	269	0	16131	58	5.5	251	4
16132	55	5	230	2	16133	58	5.5	246	0
16135	42	5	219	0	16134	47	5.5	219	–3
Average	46.6	5	238	4.1		46.3	5.5	230.5	2.5

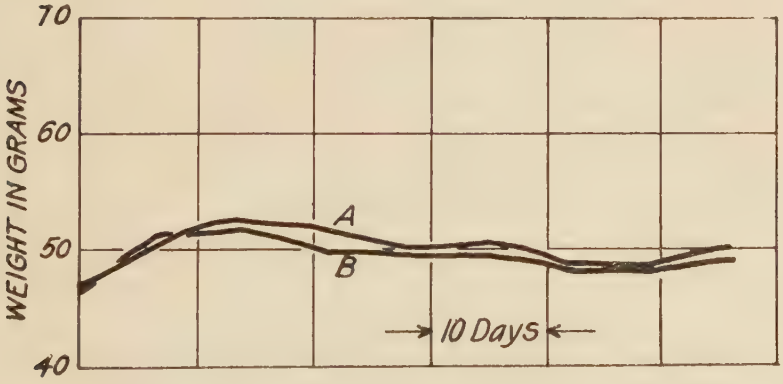

FIGURE I.

Weight curves of rats fed on basal diet together with: (A) 5 cubic centimeters of tomato juice unheated at pH = 4.28; (B) 5.5 cubic centimeters of tomato juice heated one hour at 100° C. at pH = 4.28.

Table II gives the same data for rats fed upon unheated tomato juice and upon tomato juice heated for four hours at 100° C. Figure II shows the growth curves for these rats.

TABLE II.

RATS FED ON UNHEATED TOMATO JUICE AND ON TOMATO JUICE HEATED FOUR HOURS AT 100° C. BOTH AT NATURAL ACIDITY (pH = 4.28).

Unheated					Heated Four Hours				
Rat No.	Initial Weight gms.	Dose cc.	Food Intake gms.	Gain gms.	Rat No.	Initial Weight gms.	Dose cc.	Food Intake gms.	Gain gms.
15661	42	5	242	8	15664	40	6	218	3
15680	46	5	249	1	15679	51	6	239	–5
15683	46	5	252	–1	15682	47	6	275	–3
15718	44	5	235	0	15720	40	6	230	–3
16122	36	5	237	5	15398	39	6	215	2
16129	37	5	236	6	15402	36	6	201	5
16135	42	5	219	0	15613	46	6	273	–3
16132	55	5	230	–3	15393	33	6	260	–1
Average	43.5	5	237.5	2		41.5	6	239	–0.6

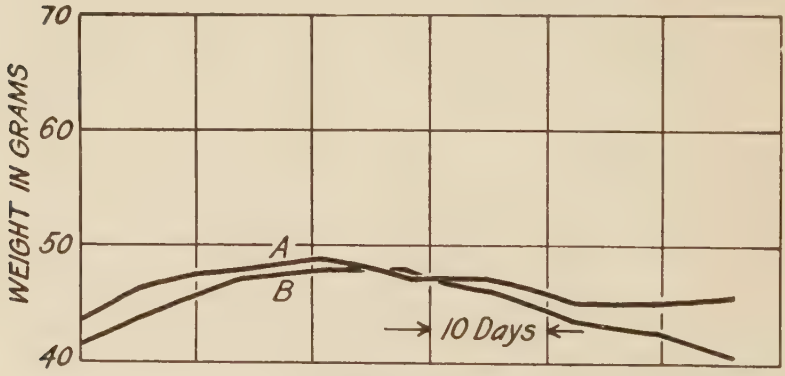

FIGURE II.

Weight curves of rats fed on basal diet together with: (A) 5 cubic centimeters of tomato juice unheated at pH = 4.28; (B) 6 cubic centimeters of tomato juice heated four hours at 100° C. at pH = 4.28.

Tomato Juice Heated for One Hour at Different Hydrogen Ion Concentrations.

The effect of heat at 100° C. for one hour was studied at effective concentrations of hydrogen ion of pH = 5.2, pH = 7.9, pH = 9.18, and pH = 10.9, respectively. In each case an effort was made merely to maintain body weight without gain during the eight weeks.

Table III shows the record of rats fed upon the basal diet together with tomato juice heated for one hour at 100° C. at an effective concentration of hydrogen ion of pH = 5.2, compared with the direct controls fed under like conditions with tomato juice heated one hour at natural acidity (pH = 4.28).

TABLE III.

RATS FED ON TOMATO JUICE HEATED ONE HOUR AT 100° C.

pH = 4.28					pH = 5.2				
Rat No.	Initial Weight gms.	Dose cc.	Food Intake gms.	Gain gms.	Rat No.	Initial Weight gms.	Dose cc.	Food Intake gms.	Gain gms.
14574	63	4	307	7	14573	63	4	279	–9
14634	40	4	220	–4	14633	42	4	244	2
14826	62	5	295	–7	14829	55	4	273	–9
15143	43	4	219	–6	15142	43	4	223	–4
14576	54	5	290	–2	14575	55	5	269	–3
14920	38	5	298	–3	14918	42	5	262	–5
14998	49	5	257	–14	15000	47	5	265	–9
15131	49	5	256	–9	15130	50	5	270	–4
15139	44	5	297	0	15140	43	5	258	1
14578	48	6	328	17	14577	49	6	321	17
14823	59	6	405	–2	14824	56	6	358	1
14617	38	6	289	17	14618	33	7	379	16
Average	48.9	5	288	–.5		48.1	5	283	–.5

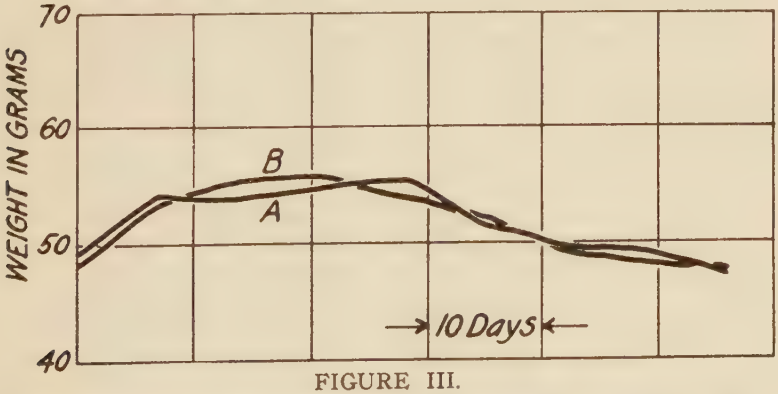

FIGURE III.

Weight curves of rats fed on basal diet together with: (A) 5 cubic centimeters of tomato juice heated one hour at 100° C. at pH = 4.28; (B) 5 cubic centimeters of tomato juice heated one hour at 100° C. at pH = 5.2.

The curves in Figure III show that the destruction due to a change of hydrogen ion concentration from pH = 4.28 to pH = 5.2 is not large enough during one hour's heating at 100° C. to be detected by our experimental method.

Table IV shows the gains in weight of rats fed upon the basal diet to which tomato juice heated one hour at 100° C., and at an effective hydrogen ion concentration of pH = 7.9 has been added. The direct controls for these animals are given in the parallel column. The growth curves (Figure IV) show 4.8 cubic centimeters of tomato juice heated for one hour at 100° C. at an effective hydrogen ion concentration of pH = 4.28 to be practically equivalent to 6.4 cubic centimeters of tomato juice heated one hour at 100° C., at an effective hydrogen ion concentration of pH = 7.9.

$$\text{Destruction} = \frac{6.4 - 4.8}{6.4} = 25 \text{ per cent.}$$

TABLE IV.

RATS FED ON TOMATO JUICE HEATED ONE HOUR AT 100° C.

	pH = 4.28					pH = 7.9			
Rat No.	Initial Weight gms.	Dose cc.	Food Intake gms.	Gain gms.	Rat No.	Initial Weight gms.	Dose cc.	Food Intake gms.	Gain gms.
14655	55	4	271	–10	14654	55	5	258	–10
14662	43	4	252	–1	14663	49	5	308	–7
15004	49	4	252	–14	14997	52	5	249	–15
14657	54	5	283	–9	15656	54	6	263	–5
14664	47	5	266	–2	14665	44	6	237	–3
14687	43	5	320	–3	14688	43	6	210	–8
14998	49	5	257	–14	14999	48	6	247	–10
15143	43	4	219	–6	15144	41	6	225	–1
15174	38	5	239	–11	15173	41	6	192	–11
15192	34	4	194	–5	15191	35	6	275	–1
14659	52	6	298	1	14658	53	7	291	–6
15139	44	5	297	0	15141	46	7	278	1
15195	32	5	237	–1	15194	35	7	236	2
15195	32	5	237	–1	15190	37	7	255	0
14659	52	6	298	1	14660	51	8	275	0
14920	38	5	298	–3	14919	38	8	286	–2
15139	44	5	297	0	15138	47	8	253	2
Average	44	4.8	266	–4.6		45	6.4	255	–4.4

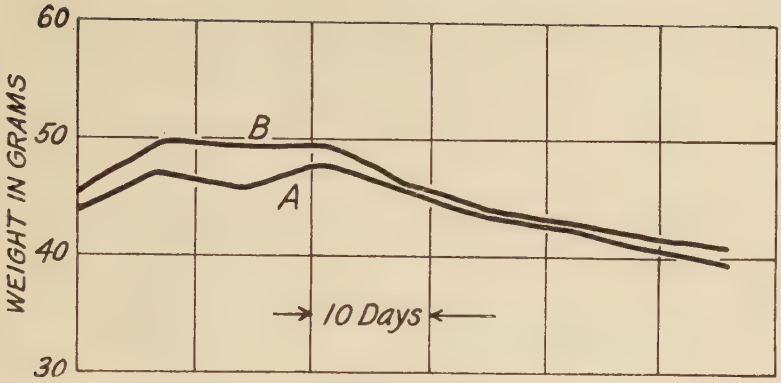

FIGURE IV.

Weight curves of rats fed on basal diet together with: (A) 4.8 cubic centimeters of tomato juice heated one hour at 100° C. at pH = 4.28; (B) 6.4 cubic centimeters of tomato juice heated one hour at 100° C. at pH = 7.9.

Table V shows the record of rats fed on the basal diet supplemented by tomato juice heated one hour at 100° C. at pH = 9.18. Since these animals were receiving dosages below maintenance, their weight curves were compared to parallel rats receiving two cubic centimeters and four cubic centimeters of tomato juice heated one hour at 100° C. Figure V shows that the growth curve for rats fed on tomato juice heated one hour at 100° C. at pH = 9.18 falls half way between the other two curves. Eight cubic centimeters of the former juice is, then, equivalent to approximately three cubic centimeters of the latter.

$$\text{Destruction} = \frac{8 - 3}{8} = 63 \text{ per cent.}$$

TABLE V.

RATS FED ON TOMATO JUICE HEATED ONE HOUR AT 100° C.*

pH = 4.28					pH = 9.18				
Rat No.	Initial Weight gms.	Dose cc.	Food Intake gms.	Gain gms.	Rat No.	Initial Weight gms.	Dose cc.	Food Intake gms.	Gain gms.
14574	63	4	307	7	15879	52	8	268	–4
14634	40	4	220	–4	15784	52	8	228	–8
15143	43	4	219	–6	15771	33	8	202	–1

* Note: For record of rats receiving two cubic centimeters of tomato juice see Table X.

14655	55	4	271	−10	15844	57	8	260	−19
14662	43	4	252	−1	15805	43	8	195	−2
15004	49	4	252	−14	15820	48	8	232	−14
15192	34	4	194	−5	15765	37	8	198	−1
					15808	44	8	235	−6
					15853	51	8	263	−14
					15883	48	8	248	−4
Average	46.7	4	245	−4.7		46.5	8	233	−7.3

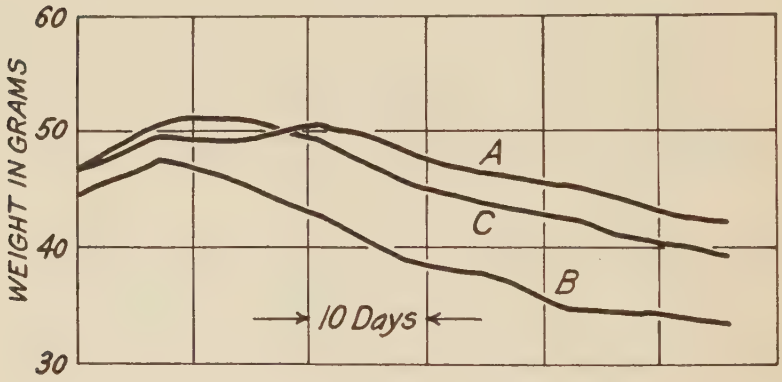

FIGURE V.

Weight curves of rats fed on basal diet together with: (A) 4 cubic centimeters of tomato juice heated one hour at 100° C. at pH = 4.28; (B) 2 cubic centimeters of tomato juice heated one hour at 100° C. at pH = 4.28; (C) 8 cubic centimeters of tomato juice heated one hour at 100° C. at pH = 9.18.

Table VI gives the initial weight, dose of tomato juice, and length of life of rats receiving in addition to the basal diet, tomato juice heated one hour at 100° C. at pH = 10.9. Compared to these in Figure VI are a group of negative controls or rats receiving only the basal diet. The average length of life for the two groups are also compared. Even though the curves do not exactly correspond, as the average length of life for rats fed on tomato juice heated at pH = 10.9 is 30.75 days and that for negative controls is 29.9 days, it is probably safe to conclude that the destruction was between 90 and 100 per cent.

TABLE VI.

RATS FED ON TOMATO JUICE HEATED ONE HOUR AT 100° C. AT pH = 10.9.

Rat No.	Initial Weight Grams	Tomato Juice cc.	Time Before Death, Days
14877	43	4	30
14870	42	5	30
14875	45	5	30
14872	37	6	30
14873	47	6	36
14907	46	6	29
15182	54	6	31
14909	42	7	30
15180	58	7	27
14911	39	8	30
15179	56	8	30
15177	60	9	36
		Average	30.75 days

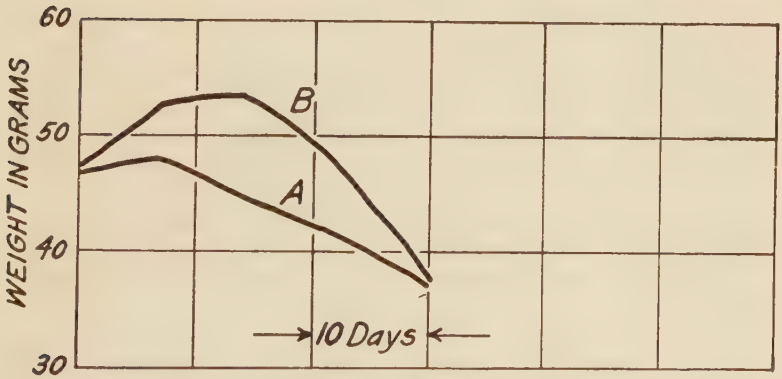

FIGURE VI.

Weight curves of rats fed on basal diet together with: (A) No tomato juice, (Average length of life = 29.9 days); (B) 6.4 cubic centimeters of tomato juice heated one hour at 100° C. at pH = 10.9, (Average length of life = 30.75 days).

Tomato Juice Heated for Four Hours at Different Hydrogen Ion Concentrations.

Tomato juice was heated for four hours at 100° C. and at pH = 5.2 and pH = 7.9 respectively.

According to the results in Table II and to the work of Sherman and Grose[8], the destruction of vitamin B in tomato

juice heated for four hours at 100° C. was found to be approximately 20 per cent. In the present investigation, tomato juice heated four hours at natural acidity (pH = 4.28) as in the work of Sherman and Grose, was compared directly with the tomato juice heated four hours at pH = 5.2. The food intake and gain in weight for rats with their positive controls, are shown in Table VII. From Figure VII, 6.9 cubic centimeters of tomato juice heated four hours at pH = 5.2 is found to be practically equivalent to 5.9 cubic centimeters of tomato juice heated for four hours at pH = 4.28.

$$\text{Destruction} = \frac{6.9 - 5.9}{6.9} = 14 \text{ per cent.}$$

TABLE VII.

RATS FED ON TOMATO JUICE HEATED FOUR HOURS AT 100° C.

	pH = 4.28					pH = 5.2			
Rat No.	Initial Weight gms.	Dose cc.	Food Intake gms.	Gain gms.	Rat No.	Initial Weight gms.	Dose cc.	Food Intake gms.	Gain gms.
15393	33	5	260	–1	15397	33	7	274	1
					15394	38	7	258	2
15398	39	5	215	2	15400	39	7	234	5
					15399	39	8	277	16
15396	36	6	240	1	15395	38	6	248	0
					15405	34	7	202	10
15402	36	6	201	5	15404	36	6	212	3
					15406	39	8	220	5
15535	44	6	255	1	15533	48	6	278	–4
					15537	41	6	235	–3
15541	44	6	261	4	15539	52	6	252	–8
					15543	47	6	240	–5
15607	39	6	255	1	15605	43	6	229	–6
15615	34	6	365	6	15609	39	6	252	–6
15613	46	6	273	–3	15611	49	6	258	–11
15720	40	6	230	–3	15616	42	6	260	–8
15664	40	6	218	3	15663	45	6	208	–2
15679	51	6	239	–5	15678	53	6	299	–2
15682	47	6	275	–3	15681	47	6	265	–7
Average	40.7	5.9	253	.6		42.2	6.9	247	–1

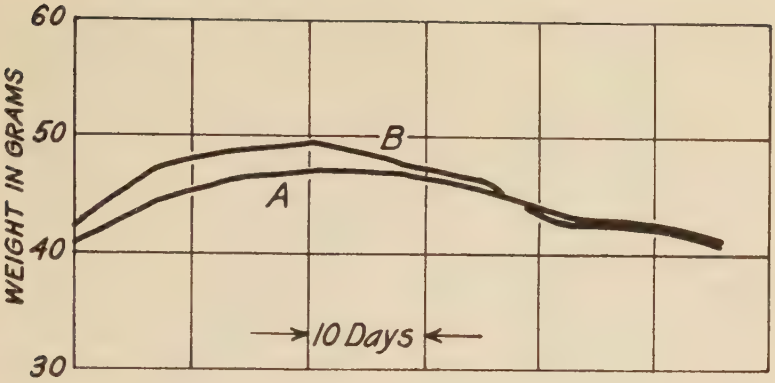

FIGURE VII.

Weight curves of rats fed on basal diet together with: (A) 5.9 cubic centimeters of tomato juice heated four hours at 100° C. at pH = 4.28; (B) 6.9 cubic centimeters of tomato juice heated four hours at 100° C. at pH = 5.2.

In Table VIII a comparison is given of the results obtained with rats fed on the basal diet together with tomato juice heated four hours at pH = 7.9 with those fed on the basal diet together with tomato juice heated for four hours at pH = 4.28. As the former group (See Figure VIII) showed a great loss in weight during the eight week period their average growth curve could not be directly compared with their direct controls. Approximating from the curves in Chart II and those of Sherman and Grose[8], 8.7 cubic centimeters of tomato juice heated four hours at 100° C. at pH = 7.9 were thought to be practically equivalent to 3.2 cubic centimeters of natural tomato juice heated four hours.

$$\text{Destruction} = \frac{8.7 - 3.2}{8.7} = 63 \text{ per cent.}$$

In order to verify this approximation, this curve (Figure VIII) was superimposed upon the curve from Figure IV which showed 63 per cent. destruction, and they were found to be approximately parallel.

TABLE VIII.

RATS FED ON TOMATO JUICE HEATED FOUR HOURS AT 100° C.

pH = 4.28					pH = 7.9				
Rat No.	Initial Weight gms.	Dose cc.	Food Intake gms.	Gain gms.	Rat No.	Initial Weight gms.	Dose cc.	Food Intake gms.	Gain gms.
15535	44	6	255	1	15534	44	8	233	–6
					15536	42	10	233	–7
15541	44	6	261	4	15540	49	8	232	–9
15607	39	6	255	1	15606	40	8	232	–8
					15608	39	10	246	–4
15613	46	6	273	–3	15612	49	8	243	–9
15615	34	6	365	6	15614	47	10	273	–7
Average	41.4	6	282	1.8		44.3	8.7	241.7	–7.1

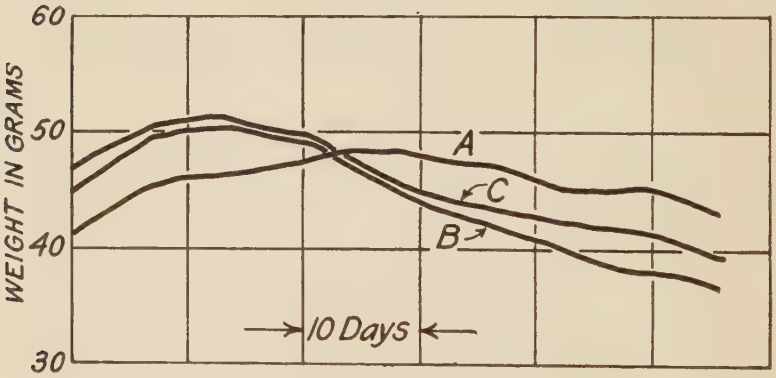

FIGURE VIII.

Weight curves of rats fed on basal diet together with: (A) 6 cubic centimeters of tomato juice heated four hours at 100° C. at pH = 4.28; (B) 8.7 cubic centimeters of tomato juice heated four hours at 100° C. at pH = 7.9; (C) 8 cubic centimeters of tomato juice heated one hour at 100° C. at pH = 9.18 (Destruction = 63 per cent.)

Effect of Oxidation Upon the Destruction of Vitamin B by Heat.

Two groups of rats of nearly equal initial weight and of similar dietary history were used for this study. One group was given the basal diet *ad libitum*, and 8 cubic centimeters of tomato juice heated in a steam bath under conditions prev-

iously described for one hour at 100° C. at pH = 9.18 while the other group received the same basal diet *ad libitum* and 8 cubic centimeters of tomato juice heated in an atmosphere of nitrogen for one hour at the same hydrogen ion concentration, (pH = 9.18). The conditions were kept as nearly alike as possible and the weight curves compared. Figure IX shows the

TABLE IX.

RATS FED ON TOMATO JUICE HEATED ONE HOUR AT 100° C. AT pH = 9.18.

Under Conditions Described in Experiment					Under Atmosphere of Nitrogen				
Rat No.	Initial Weight gms.	Dose cc.	Food Intake gms.	Gain gms.	Rat No.	Initial Weight gms.	Dose cc.	Food Intake gms.	Gain gms.
15771	33	8	202	–1	15767	35	8	159	2
15784	52	8	228	–8	15779	54	8	279	–15
15805	43	8	165	–2	15786	53	8	221	–7
15808	44	8	235	–6	15810	41	8	264	0
15820	48	8	232	–14	15823	45	8	244	–10
15853	51	8	263	–14	15852	52	8	255	–16
15879	52	8	268	–4	15881	50	8	203	–3
15883	48	8	248	–4	15886	49	8	258	–9
Average	46.4	8	230.1	–6.7		47.3	8	235.4	–7.0

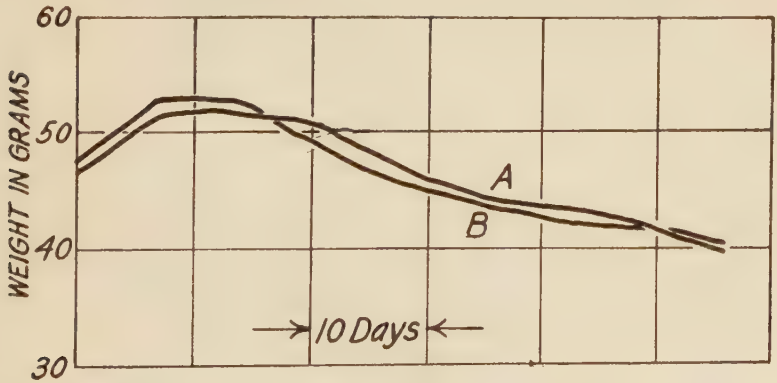

FIGURE IX.

Weight curves of rats fed on basal diet together with: (A) 8 cubic centimeters of tomato juice heated one hour at 100° C. at pH = 9.18 under conditions described in text; (B) 8 cubic centimeters of tomato juice heated one hour at 100° C. at pH = 9.18 under an atmosphere of nitrogen.

similarity in the composite weight curves for the two groups over an experimental period of eight weeks. From these results, we conclude that oxidation plays little if any part in the destruction of vitamin B under the conditions of the experiments described by Sherman and Grose[8] in this paper.

Table X shows the weekly weights of two groups of rats, one fed upon the basal diet together with only two cubic centimeters of tomato juice heated at natural acidity (pH = 4.28) according to the method of Sherman and Grose[8], and the second given the basal diet and two cubic centimeters of tomato juice heated according to the method of Quinn[16] in an atmosphere of nitrogen for one hour. Since many of these animals did not live through the experimental period there is only an approximation of the curves for the last three weeks.

The data shows plainly that exclusion of oxygen made no appreciable difference and hence that oxidation was not an appreciable factor in the work here described or in that of Sherman and Grose[8].

TABLE X.

RATS FED UPON BASAL DIET TOGETHER WITH 2 cc. TOMATO JUICE HEATED FOR ONE HOUR AT A NATURAL ACIDITY AND AT 100° C. UNDER CONDITIONS PREVIOUSLY DESCRIBED.

Rat No.	Initial Weight grams	Weight in grams at the end of Successive Weeks 1	2	3	4	5	6	7	8
15749	34	36	34	34	31	31†			
15764	40	40	34	32	31	32	30	32	30
15780	53	58	56	51	47	44	41	41	39
15787	37	38	35	33	30	27	24†		
15802	53	60	57	54	49	40	36†		
15809	36	39	38	35	31†				
15824	40	45	43	40	36	36	35	33	31
15846	55	58	53	49	42	39†			
15850	48	51	49	46	39	39	36†		
15878	52	54	56	56	51	47	40†		
15884	43	42	42	40	40	39	35	32†	
Average	44.6	47.4	45.2	42.7	38.8	37.4	34.6	34.5	33.3

(Same conditions with tomato juice heated in atmosphere of nitrogen)

Rat No.	Initial Weight grams	Weight in grams at the end of Successive Weeks 1	2	3	4	5	6	7	8
15744	34	38	36	34	33	33	34	34†	
15768	32	31	29	26†					
15783	53	58	52	51	47	43	41	39	39
15785	44	45	41	40	36	35†			
15804	45	49	47	44	44	42	40	33†	
15807	46	52	50	47	44	39	36	34†	
15819	49	55	52	52	46	44	45	42	37
15847	55	58	56	52	45	46	39	37	36
15849	52	55	54	50	43	43	36†		
15880	50	50	54	51	47	47	41	40	36
15887	45	46	46	45	40	39	37	36	34
Average	45.9	48.8	47.	44.8	42.5	41.2	38.8	36.3	36.3

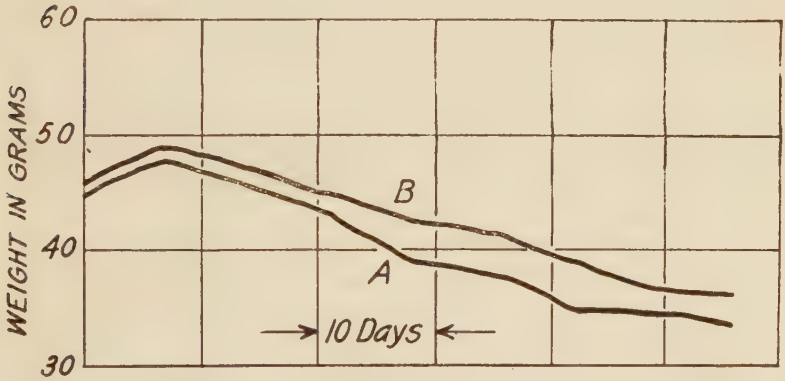

FIGURE X.

Weight curves of rats fed on basal diet together with: (A) 2 cubic centimeters of tomato juice heated one hour at 100° C. at pH = 4.28 under conditions described in text; (B) 2 cubic centimeters of tomato juice heated one hour at 100° C. at pH = 4.28 under an atmosphere of nitrogen.

DISCUSSION

Any work using animals must necessarily involve natural physiological variability as well as the ordinary errors of chemical laboratory work. Significant quantitative results can only be obtained when these individual differences are over-

come by the use of a large number of animals for each comparison. As all the litters used in this investigation were not from mothers fed upon the same stock diet, there was some possibility of slight differences in natural vitality and in storage of vitamin B. In any case, this possible storage is, according to the work of Steenbock, Sell and Jones[19], very small. The differences between litters were overcome in this investigation by using, for direct comparison, two rats from the same litter with nearly the same initial weight. In a few instances, it was necessary to compare animals taken from different litters, but in each of these cases, care was taken to use rats from mothers who had been fed upon the same stock diet.

It is not to be expected that one should be able to determine very small differences in rate of destruction of the vitamin. There was no doubt a slightly greater destruction of the vitamin B when the tomato juice was heated one hour at 100° C. at pH = 5.2 than there was when heated for one hour at 100° C. at pH = 4.28, but the difference was too small to be detected in the weight curves (Figure III). When the hydrogen ion concentration was lowered to pH = 7.9, however, the destruction was very much larger, and at the still smaller concentration of hydrogen ion of pH = 9.18, destruction increased to 63 per cent.

Under the influence of a more alkaline medium, pH = 10.9, the destruction of the vitamin B due to one hour of heating at 100° C. was almost complete. The animals must have received a very small amount of the vitamin, however, as they gained weight for the first two weeks (Figure VI), whereas the negative controls, or rats fed upon the basal diet alone, gained only a very small amount during the first week, and began to lose weight before the end of the second week. The average survival in days for the rats receiving the tomato juice in addition to the basal diet, was only one day longer than the average survival for those receiving only the basal diet. It was possible, then, to make only an approximation of the active vitamin B left in the tomato juice after heating one hour at 100° C. at pH = 10.9. The average daily allowance of this tomato juice, 6.4 cubic centimeters, appeared to be equivalent

to less than 0.5 cubic centimeter of unheated juice and as five cubic centimeters of unheated juice was found to be essential for maintenance, the destruction must have been between 90 and 100 per cent.

When the tomato juice was heated four hours at 100° C., the destruction of the vitamin B was naturally more pronounced than in one hour. Even in the acid range, at pH = 5.2, the destruction was quite significant (14 per cent.). At pH = 7.9, the destruction became so large, 63 per cent., that the rats, even when given large doses of tomato juice, were far below the maintenance level. For that reason it seemed unnecessary to attempt to measure the destruction of the vitamin B in the tomato juice heated four hours at a smaller hydrogen ion concentration.

The weight curves for all rats within one series were quite consistent except when an effort was made to determine curves far below the maintenance zone. Those rats fed only two cubic centimeters of heated tomato juice in addition to the basal diet (Figure X) yielded less regular results. Many of them died before the close of the experimental period. Those animals fed upon the basal diet to which two cubic centimeters of tomato juice heated one hour in an atmosphere of nitrogen had been added, also showed this high mortality. This high mortality, then, introduced an error into the weight curves for the last three experimental weeks. A larger percentage of the animals receiving the tomato juice heated under an atmosphere of nitrogen, survived the eight weeks' period. This could not be taken as significant evidence of difference in vitamin content, as all the rats were far below their maintenance level. There was practically no difference, however, between the weight curves of animals given the basal diet together with eight cubic centimeters of the juice of pH = 9.18 heated under an atmosphere of nitrogen, and those of animals given the basal diet and eight cubic centimeters of the same juice of pH = 9.18 heated under conditions previously described. This latter evidence may be taken as more significant as the rats all survived the eight weeks, and were not so far below maintenance.

In order to calculate the total destruction of the vitamin B in the juice, it was necessary to take into account the destruction due to heat as well as that due to change of hydrogen ion concentration. As heat applied for one hour at 100° C. at pH = 4.28 caused a destruction of about 9 per cent. and a decrease of the hydrogen ion concentration to pH = 5.2 increased the destruction less than 10 per cent., the total destruction must have been about 10 per cent. of the vitamin present in unheated juice. At pH = 7.9, the total destruction was $(91 \times .25) + 9 = 32$ or between 30 and 40 per cent., and at pH = 9.18, it was $(91 \times .63) + 9 = 66$, or between 60 and 70 per cent. When the tomato juice was heated for four hours, there was a greater destruction of the vitamin due to heat, and also, due to change of hydrogen ion concentration. At pH = 5.2, the destruction was $(80 \times .14) + 20 = 31$ or between 25 and 35 per cent., and at pH = 7.9, it was $(80 \times .63) + 20 = 70$, or between 65 and 75 per cent.

All rats which died before the end of the experimental period were autopsied at death. At the end of the eight weeks' period the surviving rats were chloroformed and autopsied. The rats which had received inadequate allowances of vitamin B (or none) were found to be very emaciated and exceedingly nervous. In the few cases of those animals receiving very small portions of tomato juice, but living through the entire eight weeks' period, distinct symptoms of polyneuritis, or paralysis in the hind legs, were found. In practically every case of rats dying from lack of vitamin B, or maintained on exceedingly small portions of vitamin B, diarrhea was found to be an active factor. The animals were very sensitive to temperature changes and showed very poor appetites varying slightly with climatic conditions. These facts made it most essential to keep animals which were to be compared under parallel conditions.

SUMMARY AND CONCLUSIONS

The destruction of vitamin B in tomato juice due to heating at 100° C. for one hour and for four hours, was determined by comparing the weight curves of rats fed upon a standard vitamin B-free diet together with unheated tomato juice and the weight curves of rats given the same diet and tomato juice previously heated one hour, or four hours, at 100° C. at pH = 4.28.

In order to determine the effect of the change of hydrogen ion concentration upon the vitamin B content of the juices, rats were fed upon a vitamin B-free diet supplemented by measured amounts of the tomato juice. The tomato juice was previously heated for one hour at 100° C. at pH = 5.2, pH = 7.9, pH = 9.18, and pH = 10.9 respectively. The effect of heat at the different hydrogen ion activities was determined by comparing the weight curves of these rats with the weight curves of rats fed upon the basal diet together with a measured amount of tomato juice heated one hour at 100° C. at pH = 4.28.

The effect of four hours heating at 100° C. at different hydrogen ion activities upon the vitamin B content of tomato juice was measured by comparing the weight curves of rats fed upon a vitamin B-free diet supplemented by tomato juice heated four hours at 100° C. at pH = 5.2 or pH = 7.9, with the weight curves of rats fed upon the basal diet together with a measured amount of tomato juice heated four hours at 100° C. at pH = 4.28.

A level which gave maintenance or a little less than maintenance starting with twenty-eight day old albino rats, was found to be most practical in determining comparable amounts of tomato juice.

The increased destruction of vitamin B over that at pH = 4.28 (natural acidity), on heating for one hour at 100° C., at pH = 5.2 was about 10 per cent.; at pH= 7.9, 20-30 per cent.; at pH = 9.18, 60-70 per cent.; and at pH = 10.9, 90-100 per cent. When the heating at 100° C. was continued for four hours, the increased destruction over that at pH = 4.28, was 10-20 per cent. at pH = 5.2, and 60-70 per cent. at pH = 7.9.

The total destruction of the vitamin B due to one hour of heating at 100° C., at the different hydrogen ion activities was as follows: at pH = 5.2, about 10 per cent.; at pH = 7.9, 30-40 per cent.; at pH = 9.18, 60-70 per cent.; and at pH = 10.9, 90-100 per cent. When the heating was continued for four hours at 100° C., the total destruction was 25-35 per cent. at pH = 5.2, and 65-75 per cent., at pH = 7.9.

The weight curves of rats fed upon the basal vitamin B-free diet and eight cubic centimeters of tomato juice heated in loosely stoppered flasks immersed to the necks in boiling water for one hour at pH = 9.18, were found to parallel the weight curves of rats fed upon the same diet and eight cubic centimeters of tomato juice heated in a flask immersed in boiling water, in an atmosphere of nitrogen, for one hour at pH = 9.18. These results indicate that oxidation was not an appreciable factor in the destruction of vitamin B under the conditions used in the investigation.

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